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TETRAETHYLAMMONIUM-SENSITIVE APICAL K^+ CHANNELS MEDIATING K^+ SECRETION BY TURTLE COLON

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SUMMARY

1. Apical membrane K^+ channels in turtle colon were identified and characterized using current fluctuation analysis.

2. Under short-circuit conditions in NaCl-Ringer solution, the power density spectrum (PDS) of the short-circuit current (I_{sc}) sometimes exhibited a clearly discernible Lorentzian component, indicating spontaneous fluctuations produced by a population of apical ion channels. The Lorentzian component had a characteristic corner frequency (f_c) which averaged $10{\cdot}2 \pm 0{\cdot}9$ Hz (mean $\pm$ S.E.M., $n = 20$).

3. The power of the spontaneous fluctuations was enhanced (S_0 increased) by manoeuvres that depolarize the apical membrane electrical potential (V_a). Discernible fluctuations were enhanced or induced by raising the serosal K^+ concentration ($[K^+]_s$ = 50–115 mM, Na^+ replacement), by clamping the transepithelial potential (V_t) to serosa-positive values, or by blocking basolateral K^+ channels with Ba^{2+}.

4. Mucosal amiloride (100 μM) attenuated the spontaneous fluctuations observed in NaCl-Ringer solution but had no effect in the presence of serosal high K^+, indicating that amiloride did *not* block the K^+-permeable channels but these channels resided in the same cells as the amiloride-sensitive Na^+ channels.

5. Raising the mucosal K^+ concentration attenuated spontaneous fluctuations.

6. In the presence of serosal high K^+ and mucosal amiloride, the spontaneous fluctuations were often accompanied by a reversed I_{sc} consistent with K^+ secretion. These conditions were used to test the effects of putative channel blockers.

7. Mucosal Ba^{2+} and tetraethylammonium (TEA^+) were effective inhibitors of the spontaneous fluctuations and the reversed I_{sc}. At a concentration of 10 mM, TEA^+ was maximally effective but the TEA^+ analogues tetramethylammonium (TMA^+) and tetrapropylammonium ($TPrA^+$) were much less effective. Mucosal Rb^+ or Cs^+ did not inhibit at a concentration of 10 mM.

8. Mucosal lidocaine (200 μM), quinidine (200 μM), or diphenylamine-2-carboxylate (DPC, 1 mM) had little or no effect on the spontaneous fluctuations and reversed I_{sc}. Quinine (100 μM), 4-aminopyridine (1 mM), and apamin (100 nM) were also without effect.

9. Mucosal TEA^+ (10 mM) abolished the active secretory K^+ flux measured in the presence of serosa-positive transepithelial potentials.

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10. These experiments identified a population of TEA^+-sensitive, apical K^+ channels which mediate active K^+ secretion in turtle colon. Sensitivity to external TEA^+ distinguishes these channels from basolateral K^+ channels in turtle colon and demonstrates similarity to apical K^+ channels in mammalian colon.

INTRODUCTION

Active K^+ secretion is a common feature of vertebrate colon (e.g. McCabe, Smith & Sullivan, 1984; Foster, Hayslett & Binder, 1984; Halm & Dawson, 1984*a*, *b*). Although it appears that K^+ exit across the apical membrane is conductive, little is known about the specific channels which mediate this process. Apical K^+ channels have been identified in mammalian colon (Wills, Zeiske & Van Driessche, 1982), but their role in K^+ secretion has not been clearly defined. Furthermore, it is not clear if apical K^+ channels are related to any of the several varieties of K^+ channels that have been identified in the basolateral membranes of colonic epithelial cells (Dawson, 1987).

Isolated turtle colon is particularly well suited for an investigation of apical K^+ channels because active K^+ secretion has been well characterized. Halm & Dawson (1984*a*, *b*) showed that the secretory K^+ flux was enhanced by serosa-positive transepithelial potentials (V_t) or serosal Ba^{2+} and was blocked by mucosal Ba^{2+} or amiloride. Those results suggested that K^+ exited the epithelial cells via a population of K^+-permeable channels in the apical membrane, although the mechanism for inhibition by amiloride was not clear. Turtle colon is also well suited for a comparison between apical and basolateral K^+ channels because several populations of basolateral K^+ channels have been identified and can be distinguished by blocker specificity (Germann, Lowy, Ernst & Dawson, 1986*b*; Chang & Dawson, 1988; Richards & Dawson, 1989).

In the present study, a population of apical K^+ channels was identified using current fluctuation analysis. We investigated the sensitivities of this channel population to amiloride and to putative K^+ channel blockers, and used the most effective blocker (TEA^+) to link this channel population to the K^+ secretory process. Some of the results have been presented in abstract form (Wilkinson & Dawson, 1990, 1991).

METHODS

Tissue preparation

The methods used to isolate turtle colons have been described by Dawson (1977). Colons were removed from pithed turtles (*Pseudemys scripta*) and stripped of smooth musculature. Pieces (0·283 cm^2) were then mounted in a perfusion chamber similar to that described by De Wolf and Van Driessche (1986). Tissues were perfused initially with NaCl-Ringer solution on both sides.

Solutions and drugs

NaCl-Ringer solution contained (mM): 112 Na^+, 2·5 K^+, 114 Cl^-, 2·5 HCO_3^-, 1 Ca^{2+} and 10 glucose. Ringer solutions with 50, 100, 112 or 115 mM K^+ were made by replacing Na^+ with K^+.

Stock solutions of amiloride (a gift from Merck, Sharpe and Dohme), barium chloride ($BaCl_2$), barium acetate, tetraethylammonium chloride (TEACl), tetramethylammonium chloride (TMACl), tetrapropylammonium chloride (TPrACl), caesium chloride (CsCl), rubidium chloride (RbCl), quinidine, quinine, apamin and 4-aminopyridine (4-AP) were made in deionized, distilled water.

Stock solutions of lidocaine and diphenylamine-2-carboxylate (DPC) were made in ethanol and dimethylsulphoxide, respectively. Aliquots of the stock solutions were added to the Ringer solution to make the final concentrations indicated.

Electrical measurements

Tissues were short-circuited ($V_t = 0$) using a low-noise voltage clamp, and the macroscopic short-circuit current (I_{sc}) was monitored continuously on a strip-chart recorder. The serosal fluid compartment was the clamp reference, and positive current was defined as cation movement from mucosa to serosa. The transepithelial slope conductance (g_t) was determined periodically by clamping V_t to -10 mV briefly (~ 1 s). Fluctuations in the macroscopic current were filtered and amplified as described by Fisher & Van Driessche (1991) and monitored continuously on an oscilloscope. To obtain a power density spectrum (PDS), filtered and amplified fluctuations were sampled at a fundamental frequency of 0·2 Hz by an analog-to-digital converter (Tecmar Labmaster) in a microcomputer (IBM PC AT). The time domain records of thirty consecutive sampling periods (sweeps) were transformed to the frequency domain by a fast Fourier transform, and the PDS was constructed by averaging the transformed records from all thirty sweeps and plotting the average values of power density (A^2 s cm^{-2}) *versus* frequency (Hz) on a double logarithmic scale. The filters, amplifiers, and computer programs were developed in the laboratory of Professor Willy Van Driessche, Leuven, Belgium (cf. Fisher & Van Driessche, 1991).

Analysis of current fluctuations

Power density spectra exhibiting a spontaneous Lorentzian component were fitted with the sum of single Lorentzian and $1/f$ components:

$$S(f) = S_0/(1+(f/f_c)^2)+S(1)/(f)^a, \quad (1)$$

where S_0 is the power of the Lorentzian low frequency plateau, f_c is the Lorentzian corner frequency, $S(1)$ is the power of the $1/f$ background noise at 1 Hz, and 'a' is the exponent which defines the slope of the $1/f$ noise. Fits to eqn (1) were made by least-squares analysis using only the data points from 0·2 to 16·5 Hz or 25·6 Hz (for $f_c > 16{\cdot}5$ Hz) because at frequencies above 30 Hz another component often contributed to the PDS (cf. Gogelein & Van Driessche, 1981*a*). In fact, most of the recorded spectra were fitted very well throughout the range between 0·2 and 94 Hz by the sum of two Lorentzian components and a $1/f$ component. The power of the apparent Lorentzian component at higher frequencies was at least an order of magnitude lower than that of the low frequency Lorentzian component, and although changes in the plateau powers of the two Lorentzian components tended to be qualitatively similar, the corner frequency of the higher frequency Lorentzian varied widely and randomly within the range 30–100 Hz, even during a single experiment. Because the significance of the apparent Lorentzian at higher frequencies was not clear, only the low frequency Lorentzian component is considered here.

The Lorentzian component was interpreted according to a two-state reaction scheme:

$$\text{Closed} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \text{Open},$$

where α and β are the rate constants for the closing and opening reactions, respectively. According to this scheme, the Lorentzian corner frequency is given by the sum of the reaction

$$2\pi f_c = \alpha+\beta. \quad (1)$$

The power of the Lorentzian plateau depends on the density of open channels (N_0), the single-channel current (i) squared, and the rate constants:

$$S_0 = 4N_0 i^2\alpha/(2\pi f_c)^2. \quad (3)$$

Single-channel current is related to the channel conductance (g) and the driving force for ion flow according to Ohm's law:

$$i = g(E_a - V_a), \quad (4)$$

where E_a is the Nernst equilibrium potential for the permeant ion and V_a is the electrical potential across the apical membrane, using the mucosal bath as reference.

Flux measurements

Transmural unidirectional fluxes of $^{42}K^+$ were measured as described by Halm & Dawson (1984*a*) except that samples were removed at 15 or 20 min intervals.

RESULTS

A spontaneous Lorentzian component in the PDS

Figure 1 shows power density spectra of the I_{sc}, recorded from four colons during bilateral perfusion with NaCl Ringer solution. Spectrum A consists mostly of $1/f$

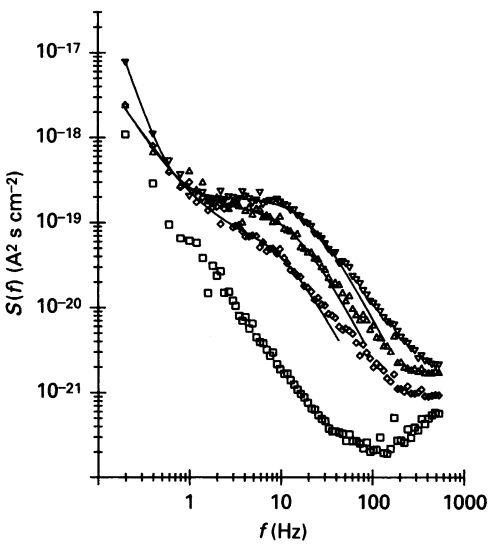

Fig. 1. Power density spectra of fluctuations in short-circuit current, recorded during bilateral perfusion of four colons (A, □; B, ◇; C, △; D, ▽) with NaCl Ringer solution. Spectrum A illustrates commonly observed background fluctuations (mostly $1/f$ noise). Spectra B–D show a clear Lorentzian component, indicating discernible current fluctuations due to spontaneously fluctuating channels in the apical membrane. The continuous curves show fits to eqn (1), using the data points in the range 0·2–16·5 or 25·6 Hz. Values for the Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
B	10·2	6·8
C	13·4	14·6
D	19·7	19·6

noise. In contrast, spectra B, C and D exhibit a clearly discernible Lorentzian component. Out of twenty-nine colons used in this study, twenty (69%) initially exhibited a measurable spontaneous Lorentzian component in the PDS. The mean f_c of the Lorentzian component was $10{\cdot}2 \pm 0{\cdot}9$ Hz, with a range from 6·0 to 19·7 Hz, and the average value of S_o was $8{\cdot}8 \pm 2{\cdot}1 \times 10^{-20}$ A² s cm^{-2}, with a range between 1·45 and $41{\cdot}3 \times 10^{-20}$ A² s cm^{-2}. Because this Lorentzian component appeared in the absence

of any channel blockers, we interpreted it as evidence of spontaneous fluctuations in a population of ion channels (cf. Gögelein & Van Driessche, 1981*a*). We postulated further that the fluctuations arose from apical ion channels because the apical membranes of tight epithelia generally contribute more resistance than the

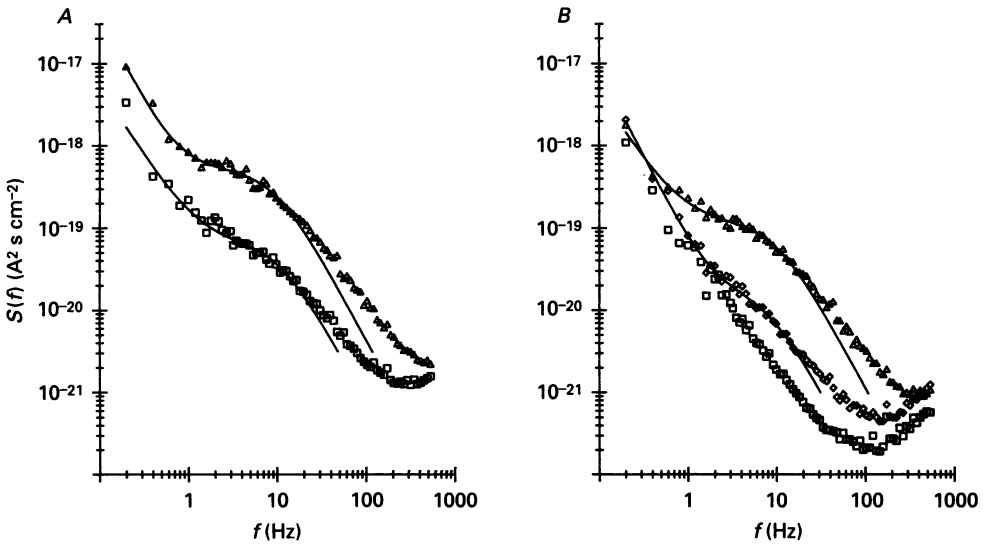

Fig. 2. Amplitudes of the spontaneous current fluctuations were increased by manoeuvres which depolarize the apical membrane, suggesting that the fluctuations were due to apical K^+ exit. *A*, the PDS exhibited a Lorentzian component during perfusion of a tissue with NaCl-Ringer solution (□). Raising the serosal K^+ concentration, $[K^+]_s$, to 112 mM (△) by Na^+ replacement substantially enhanced the power of the spontaneous fluctuations (S_o increased). The Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
NaCl-Ringer solution	10·7	6·3
$[K^+]_s$ = 112 mM	9·2	50·9

B, during perfusion of a different tissue with NaCl-Ringer solution, a Lorentzian component was not discernible. However, a Lorentzian component was discernible when the transepithelial potential (V_t) was clamped to 30 mV, serosa positive (◇), or when $[K^+]_s$ was raised to 112 mM (△). The Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
V_t = 30 mV (serosa positive)	7·9	1·6
$[K^+]_s$ = 112 mM	9·8	11·1

basolateral membranes under these conditions (Thompson, Suzuki & Schultz, 1982; Tang, Abramcheck, Van Driessche & Helman, 1985). Our initial experiments were designed to identify the specific source of the spontaneous fluctuations.

The spontaneous Lorentzian reflected fluctuations in a population of apical K^+ channels

The two most likely sources of apical current fluctuations were Na^+ entry and K^+ exit. These two alternatives were easily discriminated by measuring the current

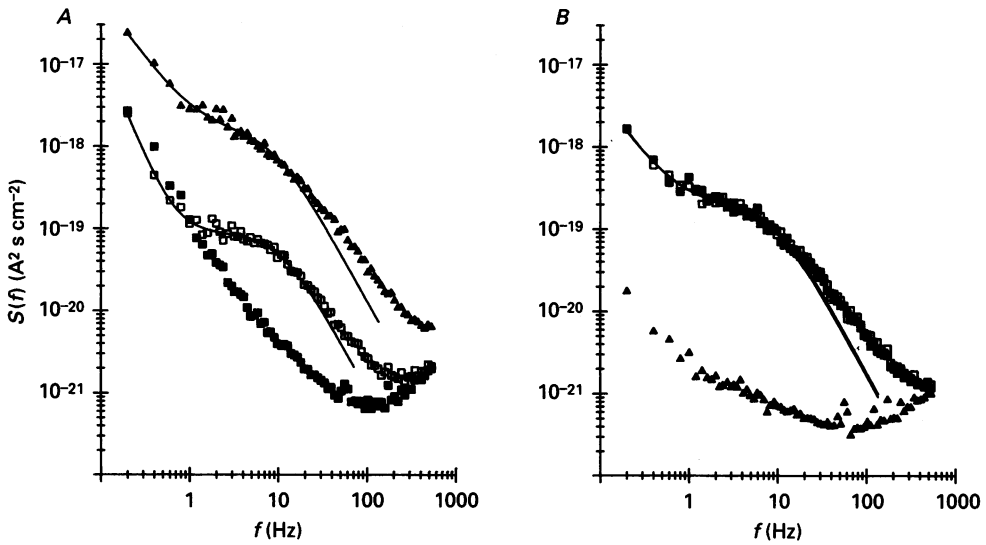

Fig. 3. Mucosal amiloride did *not* block the channels mediating apical K^+ exit. *A*, the Lorentzian component observed in NaCl-Ringer solution (□) was attenuated by 100 μM mucosal amiloride (■), but raising $[K^+]_s$ to 112 mM (▲) in the continued presence of amiloride increased S_o above the control value. The Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
NaCl-Ringer solution	11·0	8·8
$[K^+]_s$ = 112 mM, amiloride (mucosal)	9·0	141·0

B, in another colon, the Lorentzian component observed in the presence of 112 mM serosal K^+ (□) was *not* affected by 100 μM mucosal amiloride (■) but was subsequently attenuated by raising the mucosal K^+ concentration, $[K^+]_m$, to 112 mM in the continued presence of amiloride (▲, $[K^+]_m = [K^+]_s$). Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
$[K^+]_s$ = 112 mM	9·6	18·9
$[K^+]_s$ = 112 mM, amiloride (mucosal)	9·2	18·5

fluctuations before and after depolarizing V_a. Because of the opposite orientations of E_{Na} and E_K across the apical membrane, this manoeuvre was expected to decrease the driving force for Na^+ entry and increase the driving force for K^+ exit (eqn (4)), thereby increasing the amplitude of current fluctuations due to K^+ exit (eqn (3)).

Figure 2 compares power density spectra recorded before and during perfusion of the colon's basolateral surface with high-K^+-Ringer solution, a manoeuvre which depolarizes V_a in short-circuited colonic epithelia (Thompson *et al.* 1982). When

spontaneous fluctuations were discernible initially, raising the serosal K^+ concentration ($[K^+]_s$) from 2·5 to 112 mM increased the amplitude of the fluctuations. This is illustrated in Fig. 2*A* by a substantial increase in S_o. Enhanced values of S_o ranged between 2·5 and 44 times the control values (mean = 10 ×) in thirteen tissues. Changes in f_c ranged from −2 to +7 Hz (mean = +2 Hz), indicating only slight changes in the gating rate constants of the channels. Hence, the observed increase in S_o was consistent with an increase in single-channel current (i) due to depolarization of V_a (eqns (3) and (4)). When spontaneous fluctuations were not discernible initially, raising $[K^+]_s$ induced them. This is illustrated in Fig. 2*B* by the appearance of a Lorentzian component in the PDS where initially none was detected. Clamping the transepithelial electrical potential (V_t) to serosa-positive values is also expected to depolarize V_a (Thompson *et al.* 1982). Figure 2*B* shows that clamping the transepithelial potential (V_t) to 30 mV, serosa positive, induced a Lorentzian component with a plateau power lower than that induced subsequently in the same tissue by serosal high K^+. The net macroscopic current (I_{sc}) was always positive under short-circuit conditions in bilateral NaCl-Ringer solution and always decreased in response to raising $[K^+]_s$ or to clamping V_t serosa positive, consistent with a decrease in Na^+ absorptive current and/or an increase in K^+ secretory current. Hence, the concomitant increase in the Lorentzian plateau power (S_o) suggested strongly that the spontaneous current fluctuations were due to K^+ exit through apical channels.

Amiloride does not block apical K^+ *channels*

Current fluctuations were measured in the absence and presence of 100 μM amiloride, a dose which completely blocks the apical Na^+ channels. During bilateral perfusion of tissues with NaCl-Ringer solution, mucosal amiloride attenuated the spontaneous current fluctuations to near or below the level of detection ($n = 9$). This is illustrated in Fig. 3*A* by the apparent abolition of the Lorentzian component in the PDS. However, subsequently raising $[K^+]_s$ to 112 mM in the continued presence of mucosal amiloride restored the Lorentzian component, increasing S_o above the initial value. This suggested that the effect of amiloride on the current fluctuations was attributable to hyperpolarization of V_a (Thompson *et al.* 1982) and the consequent reduction of the driving force for apical K^+ exit. This interpretation was supported by other experiments in which mucosal amiloride had little or no effect on the spontaneous current fluctuations in the presence of high serosal K^+ (Fig. 3*B*). Amiloride does not cause significant hyperpolarization of V_a in tight, Na^+-absorbing epithelia bathed with serosal high K^+ (Tang *et al.* 1985). In contrast, the Lorentzian component was markedly attenuated when the K^+ concentration gradient across the apical membrane was reduced by raising the mucosal K^+ concentration to 112 mM in the presence of mucosal amiloride. In other experiments using serosal high K^+ and mucosal amiloride (not shown), clamping V_t mucosa positive attenuated the spontaneous current fluctuations. These results suggest that the spontaneous current fluctuations were due to K^+ secretion through a population of apical channels separate from the amiloride-sensitive Na^+ channels but residing in the same cells.

The apical K^+ channels were blocked by external Ba^{2+} and TEA^+

In the presence of 100 μM mucosal amiloride and 112 mM serosal K^+, the apical K^+ channels could be studied in the absence of functional Na^+ channels and the effects of blockers could be tested by observing the effects on the Lorentzian component in

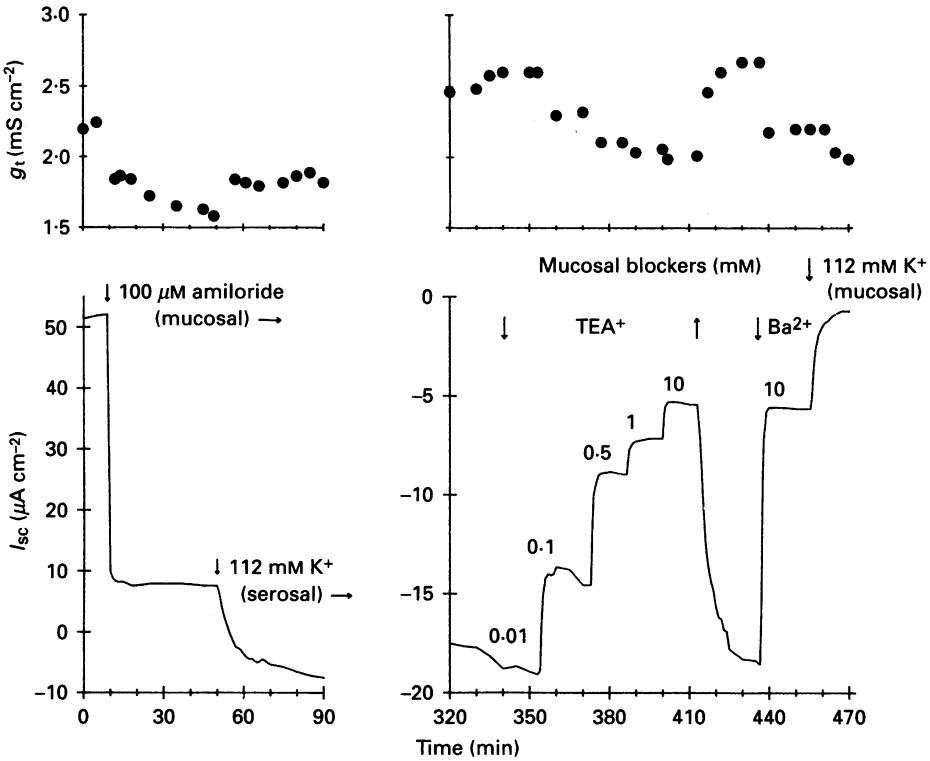

Fig. 4. Induction of a reversed I_{sc} consistent with K^+ secretion and inhibition by TEA^+ and Ba^{2+}. Mucosal amiloride (100 μM) abolished the positive I_{sc} that was due to Na^+ absorption through apical Na^+ channels, then raising $[K^+]_s$ to 112 mM in the continued presence of amiloride induced a negative, or reversed, I_{sc}. The reversed I_{sc} increased gradually for several hours (note break in the time scale and change in the current scale). Mucosal TEACl inhibited the reversed I_{sc} at concentrations greater than 10 μM and up to 10 mM. The I_{sc} recovered upon wash-out of TEA^+ and was subsequently inhibited by 10 mM $BaCl_2$. In the continued presence of $BaCl_2$, raising $[K^+]_m$ to 112 mM abolished the I_{sc}.

the PDS. In some cases, the macroscopic current (I_{sc}) reversed, consistent with a measurable K^+ secretory current, and the reversed I_{sc} provided an additional measure of blocker efficacy. An example of the protocol used in sixteen experiments is shown in Fig. 4. Amiloride (100 μM) was added to the mucosal perfusate to completely block the apical Na^+ channels and, hence, abolish the positive I_{sc} due to active Na^+ absorption. A small, amiloride-insensitive, positive I_{sc} was often observed and is consistent with active HCO_3^- secretion (Dawson, 1977). Raising the serosal K^+

concentration to 112 mM (to induce easily measurable spontaneous current fluctuations) caused I_{sc} to reverse so that the effects of putative K^+ channel blockers could be evaluated by means of changes in I_{sc} as well as changes in the PDS.

Figures 4 and 5*A* and *B* show the results of a single experiment that demonstrates blockade of the apical K^+ channels by TEA^+ and Ba^{2+}. Although the magnitudes of the reversed I_{sc} and the Lorentzian plateau (S_0) values were exceptionally high in that experiment, the results were qualitatively identical to those obtained in other experiments with initially lower values of I_{sc} and S_0. Mucosal TEA^+ reversibly decreased the reversed I_{sc} and the amplitudes of the spontaneous fluctuations at concentrations greater than 10 μM and up to 10 mM ($n = 10$). The inhibition by TEA^+ was concentration dependent, as shown in Figs 4 and 5*A* by progressive reductions in the reversed I_{sc} and the Lorentzian plateau power (S_0) with increasing TEA^+ concentrations. In the presence of 10 mM TEA^+, the Lorentzian component was no longer discernible in the PDS. These results are consistent with progressive reductions in the number of open channels (eqn (3)) due to increasing blocker concentration.

Mucosal Ba^{2+} reversibly decreased the reversed I_{sc} and the amplitudes of the spontaneous fluctuations at concentrations between 500 μM and 20 mM ($n = 10$), and the inhibition was concentration dependent (not shown). In the experiment shown in Fig. 4, 10 mM Ba^{2+} inhibited the reversed I_{sc} to the same level attained previously with 10 mM TEA^+, and the remaining I_{sc} was abolished by raising the mucosal potassium concentration ($[K^+]_m$) to 112 mM ($[K^+]_m = [K^+]_s$). As shown in Fig. 5*B*, the spontaneous fluctuations reappeared after washing out TEA^+, and the amplitudes were reduced by 10 mM mucosal Ba^{2+} and then further attenuated by raising $[K^+]_m$ to 112 mM. A comparison of the Lorentzian plateau (S_0) values observed in the presence of 10 mM Ba^{2+} (Fig. 5*B*) and 10 mM TEA^+ (Fig. 5*A*) indicates that blockade of this channel population by Ba^{2+} was less effective than blockade by TEA^+. This result is consistent with lower affinity of the channels for Ba^{2+} or greater voltage sensitivity of the blockade by divalent Ba^{2+} (cf. De Wolf & Van Driessche, 1986) under the depolarized conditions employed (high serosal K^+).

The ability of quaternary ammonium compounds to block this channel population was further investigated in two experiments by comparing the efficacies of TEA^+ and two other compounds, TMA^+ and $TPrA^+$. In the experiment shown in Fig. 5*C*, the apical surface of a single tissue was exposed to 10 mM TEA^+, 10 mM TMA^+, and 10 mM $TPrA^+$, each in succession (wash in, wash out). Although 10 mM TEA^+ reversibly attenuated the spontaneous fluctuations to an undetectable level (cf. Fig. 5*A*), TMA^+ and $TPrA^+$ were comparatively ineffective at the same concentration.

Blockers of basolateral K$^+$ channels did not block the apical K$^+$ channels

Quinidine blocks a distinct, swelling-induced population of basolateral K^+ channels in turtle colon epithelium and is maximally effective at a concentration of 200 mM (Germann *et al.* 1986*b*). Lidocaine also blocks these channels at concentrations of 200 μM or less (Richards & Dawson, 1986) and induces fluctuations that are visible in the power density spectrum of colons with amphotericin-permeabilized apical membranes (Dawson, Van Driessche & Helman, 1988). We investigated possible

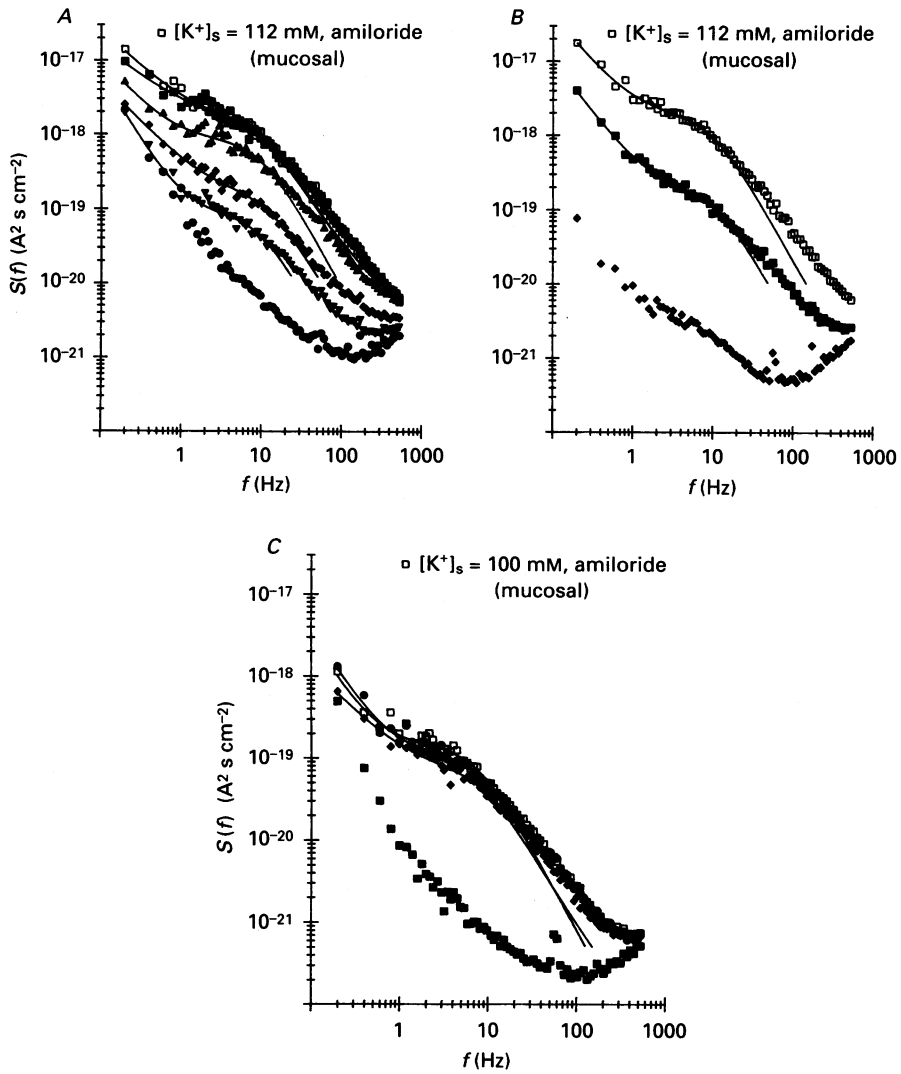

Fig. 5. Mucosal TEA^+ and Ba^{2+} blocked the channels mediating apical K^+ exit (same experiment as in Fig. 4). *A*, TEA^+ attenuated the Lorentzian component in a dose-dependent fashion. The Lorentzian parameters were:

$[TEA^+]_m$ (mM)		f_c (Hz)	S_o (10^{-20} A^2 s cm^{-2})
0	(□)	12·0	127
0·01	(■)	10·2	133
0·1	(▲)	10·1	79
0·5	(◆)	13·1	13·6
1	(▼)	9·8	8·1
10	(●)	—	—

B, the Lorentzian component reappeared after wash-out of TEA^+ then was attenuated partially by 10 mM mucosal Ba^{2+} (■) and completely by raising $[K^+]_m$ to 112 mM (◆) in the continued presence of $BaCl_2$. The Lorentzian parameters were:

blockade of the TEA^+-sensitive, apical K^+ channels by quinidine ($n = 4$) and lidocaine ($n = 3$). In the experiment shown in Fig. 6*A*, the apical surface of a single tissue was exposed in succession to 10 mM TEA^+, 200 μM lidocaine, and 200 μM quinidine (wash in, wash out). The spontaneous fluctuations were reversibly attenuated to an undetectable level by 10 mM TEA^+ (cf. Fig. 5*A* and *C*) but were unaffected by 200 μM lidocaine and only slightly reduced by 200 μM quinidine. Neither of these blockers induced additional Lorentzian components in the observed PDS.

Diphenylamine-2-carboxylate (DPC) blocks a separate population of basolateral K^+ channels in turtle colon (Richards & Dawson, 1989) and is maximally effective at a concentration of 1–2 mM. Possible blockade of the apical K^+ channels by DPC was tested in three experiments. Figure 6*C* shows the results of one experiment in which the apical surface of a single tissue was exposed to 1 mM DPC after first verifying that the spontaneous current fluctuations were inhibitable by TEA^+. The spontaneous fluctuations were reversibly attenuated to an undetectable level by 10 mM TEA^+ but were not affected by 1 mM DPC, and DPC did not induce additional fluctuations within the range of the observed PDS. Thus, the results shown in Fig. 6 demonstrate that the apical K^+ channels are pharmacologically distinct from two separate populations of K^+ channels in the basolateral membrane.

Several other putative K^+ channel blockers were tested in an attempt to identify other pharmacological probes for the apical K^+ channels. Quinine, the stereoisomer of quinidine, had no effect at a concentration of 100 μM; 4-aminopyridine (1 mM) and apamin (10 nM) were also without effect. At a concentration of 10 mM, neither mucosal Cs^+ nor Rb^+ significantly affected the spontaneous fluctuations, although 112 mM mucosal Rb^+ (Na^+ replacement) significantly reduced the amplitudes (not shown).

TEA⁺-sensitive apical K⁺ channels mediate active K⁺ secretion

To determine if the apical K^+ channels identified by fluctuation analysis mediate active K^+ secretion, we measured $^{42}K^+$ fluxes across pieces of isolated turtle colon that were voltage clamped such that the transepithelial potential difference was near the spontaneous, serosa-positive value. This condition is optimal for K^+ secretion (Halm & Dawson, 1984*b*) and most closely approximates the physiological situation.

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	f_c (Hz)	S_o (10^{-20} A^2 s cm^{-2})
TEA^+ wash-out	9·9	171
10 mM mucosal Ba^{2+}	11·6	16·6

C, in another colon, the Lorentzian component observed with $[K^+]_s$ = 100 mM was barely affected by 10 mM mucosal $TPrA^+$ (●) and was inhibited only slightly by 10 mM mucosal TMA^+ (◆), although it was attenuated by 10 mM TEA^+ (■). The Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A^2 s cm^{-2})
$[K^+]_s$ = 100 mM	7·6	14·2
10 mM $TPrA^+$ (mucosal)	8·6	10·9
10 mM TMA^+ (mucosal)	9·5	6·3

Four pieces of tissue were obtained from each of four colons, and secretory $^{42}K^+$ fluxes were measured across three pieces while absorptive flux was measured across the fourth. The results of these experiments are shown in Fig. 7. In tissue sections that exhibited substantial rates of K^+ secretion (three of twelve), mucosal TEA^+

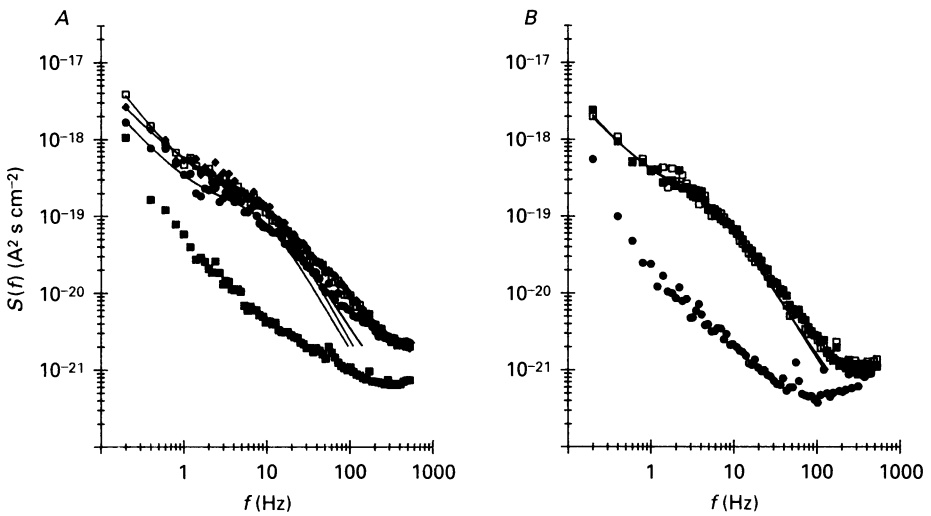

Fig. 6. Blockers of identified basolateral K^+ channels did *not* block the apical K^+ channels. *A*, the power of the Lorentzian component observed in the presence of 112 mM serosal K^+ and 100 μM mucosal amiloride (□) was *not* changed appreciably by 200 μM mucosal lidocaine (◆) or 200 μM mucosal quinidine (●) but was attenuated by 10 mM mucosal TEA^+ (■). The Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
$[K^+]_s$ = 112 mM	9·8	21·2
200 μM lidocaine (mucosal)	9·7	21·3
200 μM quinidine (mucosal)	9·9	13·5

Note that at 200 μM, either lidocaine or quinidine completely blocks a population of swelling-induced K^+ channels in the basolateral membrane of turtle colon epithelium. *B*, the power of the Lorentzian component observed in another colon under the same conditions was *not* changed by 1 mM mucosal DPC (■) but was attenuated by 10 mM mucosal TEA^+ (●). The Lorentzian parameters were:

	f_c (Hz)	S_o (10^{-20} A² s cm^{-2})
$[K^+]_s$ = 112 mM	6·4	17·3
1 mM DPC (mucosal)	6·2	17·1

Note that at 1 mM, DPC completely blocks a separate population of basolateral K^+ channels in turtle colon epithelium.

(10 mM) reduced the serosa to mucosa $^{42}K^+$ flux to the level observed in sections lacking TEA^+-sensitive K^+ transport, and subsequent addition of mucosal Ba^{2+} (10 mM barium acetate) produced no additional inhibition. Mucosal TEA^+ and Ba^{2+} had no effect on initially low secretory K^+ fluxes or on absorptive fluxes. We presume that the residual net flux in the presence of TEA^+ reflects passive diffusion of $^{42}K^+$

via the paracellular shunt (Halm & Dawson, 1984*b*). These results indicated that TEA^+-sensitive, apical K^+ channels were the route of active K^+ secretion in turtle colon.

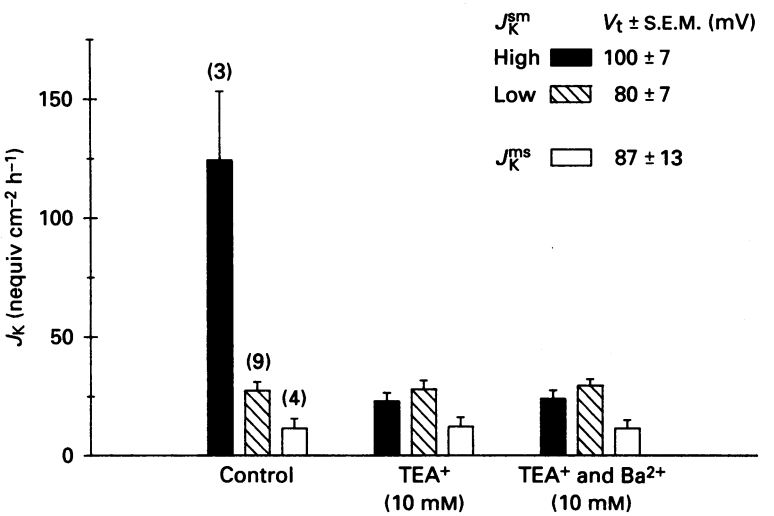

Fig. 7. K^+ secretion was inhibited by mucosal TEA^+, and mucosal Ba^{2+} produced no further inhibition, indicating that a single population of TEA^+- and Ba^{2+}-sensitive apical K^+ channels mediates K^+ secretion. Unidirectional $^{42}K^+$ fluxes were measured across pieces of colon (four colons, four pieces per colon) bathed bilaterally in NaCl-Ringer solution and voltage clamped to the spontaneous open-circuit potentials ($V_t = 110$ mV, serosa positive). After an initial control period, 10 mM TEACl was added to the mucosal bath, then 10 mM barium acetate was added as well. TEA^+ reduced the rate of K^+ secretion (J_K^{sm}) in tissues with high rates to the level exhibited by tissues with initially low rates. The rate of active K^+ absorption (J_K^{ms}) was low initially and was not changed. Numbers above bars indicate the number of tissues tested.

DISCUSSION

TEA^+ sensitivity distinguishes the apical K^+ channels from basolateral K^+ channels

In this paper we present evidence that a population of apical channels which mediates active K^+ secretion in turtle colon is blocked by TEA^+ but not by blockers of several distinct populations of basolateral K^+ channels. It now appears that the membranes of epithelial cells are inhabited by several distinctly different types of K^+ channels, and that each may have a different function in the cell (Dawson, 1987; Geibel, Zweifach, White, Wang and Giebisch, 1990). Until the molecular structures of epithelial K^+ channels are revealed, the process of distinguishing one population from another is necessarily based on a comparison of properties such as ion selectivity, gating, and blocker specificity. It seems unlikely that a general pharmacology of epithelial K^+ channels will emerge, but within a single epithelial tissue or cell type it may be possible to find relatively specific pharmacological probes which can be used to identify specific K^+ channel populations with specific transport functions.

At least three populations of K^+ channels have been identified in the basolateral membrane of turtle colon epithelium, and these can be distinguished at least partially on the basis of blocker specificity (Dawson, 1987). One population of basolateral K^+ channels appears to mediate the recycling of K^+ necessary to maintain electrogenic Na^+ absorption (Germann, Ernst & Dawson, 1986*a*) and is blocked by DPC (Richards & Dawson, 1989) but *not* by serosal TEA^+ up to 20 mM (Germann *et al.* 1986*b*). Another distinct population appears to mediate swelling-induced basolateral K^+ exit (Germann *et al.* 1986*a*) and is blocked by quinidine and lidocaine (Richards & Dawson, 1986) but *not* by up to 20 mM TEA^+ (Germann *et al.* 1986*b*). Blockade of these channels by lidocaine also induces current fluctuations that produce a Lorentzian component in the PDS (Dawson, Van Driessche & Helman, 1988). A basolateral population of Ca^{2+}-activated K^+ channels is blocked by internal TEA^+ but *not* by external (serosal) TEA^+ (Chang & Dawson, 1988). The apical channels that mediate K^+ secretion appear to be another distinct population of K^+ channels in turtle colon epithelial cells, distinguished both by lack of sensitivity to blockers of the identified basolateral K^+ channels and by sensitivity to external TEA^+.

TEA^+-sensitive K^+ channels mediate K^+ secretion in vertebrate colon

TEA^+ sensitivity appears to be a common feature of apical K^+ channels mediating K^+ secretion in mammalian colons. In rat colon, mucosal TEA^+ inhibits an apical conductance in both proximal (Sandle & McGlone, 1987) and distal segments (Sandle, Foster, Lewis, Binder & Hayslett, 1985; Binder, McGlone & Sandle, 1989) and also inhibits K^+ secretion in the distal segment (Edmonds & Willis, 1988; Sweiry & Binder, 1989). Mucosal TEA^+ also inhibits K^+ secretion in distal colon of the guinea-pig (Ishida & Suzuki, 1987). In rabbit descending colon, mucosal TEA^+ inhibits an apical conductance (Wills, 1985), spontaneous fluctuations of apical K^+ channels (Wills *et al.* 1982), and a reversed I_{sc} due to K^+ secretion (Halm & Frizzell, 1986). Although not as well documented, TEA^+-sensitive apical K^+ channels have also been reported for proximal and descending segments of human colon (Wills & Zweifach, 1987). Hence, TEA^+ sensitivity may be a common feature of apical K^+ channels that mediate K^+ secretion in vertebrate colon, and the TEA^+ binding site may, therefore, prove to be useful in molecular identification of these channels (cf. MacKinnon & Yellen, 1990).

Despite similar blocker specificity, the apical K^+ channels in turtle colon and in mammalian colons may be expressed by functionally different cell types. In turtle colon, amiloride inhibits K^+ secretion (Halm & Dawson, 1984*a*) and the spontaneous current fluctuations arising from the apical K^+ channels, but only under conditions in which amiloride is expected to substantially reduce the electrochemical driving force for K^+ secretion across the apical membrane (Fig. 3). That is, under short-circuit conditions in symmetrical NaCl-Ringer solutions, blockade of apical Na^+ channels in Na^+-transporting epithelial cells causes the fractional resistance of the apical membrane to increase to near unity, causing the apical membrane potential (V_a) to hyperpolarize toward the substantially negative value of E_K across the basolateral membrane. Because in symmetrical Ringer solution the values of E_K across the apical and basolateral membranes are the same, hyperpolarization of V_a toward E_K abolishes the net electrochemical driving force for K^+ exit across the

apical membrane. In contrast, raising the serosal K^+ concentration above 100 mM causes E_K across the basolateral membrane to approach zero such that blockade of the apical Na^+ channels does not cause V_a to hyperpolarize but instead causes V_a to remain near zero, so a substantial electrochemical driving force (E_K) favourable to apical K^+ exit remains. Hence, the observed inhibition of K^+ secretion by amiloride

TABLE 1. Corner frequencies (f_c) of apical K^+ channel fluctuations

	f_c (Hz)	Temperature (°C)
Human colon	14	37
Rabbit colon	15	37
Turtle colon	10	20
Necturus gall-bladder	3	20
Frog skin	81	20

indicates that the apical K^+ channels mediating K^+ secretion in turtle colon are expressed in the same cells that carry out active Na^+ absorption.

In contrast, numerous studies of mammalian colons (Frizzell, Koch & Schultz, 1976; McCabe *et al.* 1984; Foster *et al.* 1984; Plass, Gridl & Turnheim, 1986) have shown that amiloride does *not* inhibit K^+ secretion, leading to the suggestion (Halm & Frizzell, 1986) that K^+ secretion in these tissues could be a property of crypt cells, which do not express apical Na^+ channels and carry out salt secretion rather than absorption. Direct evidence for K^+ secretion by crypt cells in guinea-pig colon has been obtained recently by means of electron microprobe analysis (Halm & Rick, 1992), but an additional component of K^+ secretion from surface cells in rabbit descending colon cannot be excluded (Duffey & Devor, 1990). Hence, apical K^+ channels may be expressed in the secretory crypt cells as well as in the primarily absorptive surface cells of mammalian colons.

Noise analysis of apical K^+ *channels*

The analysis of spontaneous K^+ current fluctuations provided a glimpse into the properties of apical K^+ channels in turtle colon. The average corner frequency of the Lorentzian component ($f_c = \sim 10$ Hz) is an identifying 'fingerprint' related to the opening and closing kinetics of the channels. As indicated by eqn (2), the corner frequency establishes limits for the values of the individual closing and opening rate coefficients (α and β), providing an impression of the channel kinetics that would be expected in a single-channel recording. In general, fluctuations with a low corner frequency correspond to channels that undergo relatively few state transitions per unit time while a high corner frequency corresponds to channels that undergo rapid transitions. This notion provides a basis for comparison with apical K^+ channels described in other epithelia, including rabbit (Wills *et al.* 1982) and human (Wills, Alles, Sandle & Binder, 1984) descending colon, *Necturus* gall-bladder (Gögelein & Van Driessche, 1982*a*, *b*), and frog skin (Van Driessche & Zeiske, 1980). Table 1 lists the average corner frequencies of spontaneous current fluctuations arising from these K^+ channel populations. The corner frequencies observed in colons of the turtle, rabbit, and human are similar, but are higher than that observed in *Necturus* gall-bladder and much lower than that observed in frog skin. This impression can be put

in somewhat more concrete form by combining eqn (2) with the definition of closed probability ($P_c = \alpha/\alpha+\beta$) to obtain values of mean open time (T_o):

$$T_o = 1/\alpha = 1/(2\pi f_c P_c).$$

This equation emphasizes that for a measured value of f_c, limiting values of T_o can be calculated for any range of P_c. For example, if $0{\cdot}1 < P_c < 0{\cdot}9$ and the average corner frequency is 10 Hz, the mean open time for apical K^+ channels in turtle colon must be in the range 18–159 ms. For the channels in rabbit and human colons ($f_c \sim 15$ Hz), these mean open times must fall within a similar range. In contrast, the mean open times for the channels in *Necturus* gall-bladder must fall within an overlapping but clearly different range of longer times between 58 and 526 ms, and the mean open times of the channels in frog skin must fall within another range of clearly shorter times between 2 and 20 ms.

The analysis of spontaneous current fluctuations proved to be a particularly useful tool for characterizing apical K^+ channels in turtle colon because the spontaneous gating kinetics of these channels produced a Lorentzian component in the PDS. Ideally, the conduction and gating properties of these channels could be determined from single-channel recordings using the patch-clamp technique, but this approach is not generally feasible due to difficulties in sealing patch pipettes to apical membranes of intact epithelia. Alternatively, an analysis of *blocker-induced* current fluctuations would have yielded values for single-channel current and the density of these channels in the apical membrane, but none of the identified blockers induced discernible fluctuations within the range of the observed power density spectrum. Hence, the presence of spontaneous current fluctuations provided the only way presently available to characterize the apical K^+ channels in turtle colon. In fact, the use of spontaneous fluctuations to determine the blocker specificity of the channels was an advantage because potential interactions between a fluctuation-inducing blocker and other putative blockers were avoided.

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